

Team iGEM UFSCar-Brasil

PROTOCOL FOR PEG 6000 SOLUTIONS PREPARATION

1. Prepare a stock solution of 500mM Sodium Acetate in water.
2. Prepare a stock solution of 100mM zinc chloride in water.
3. Calibrate the corresponding amounts of the final polyethylene concentration of osmolyte in solution using a precision balance.
4. Prepare a stock solution of glycerol 2% vol/vol.
5. Prepare a stock solution of previously grown bacteria harvested by centrifugation at 1% (wt/vol).
6. Autoclave stock solutions of first 4 steps.
7. Polyethylene glycol solutions should be autoclaved in separated containers.
8. In a sterile laminar flow, using appropriated micropipettes and pipettes, transfer volumes of each component to a common container corresponding to the appropriate amount of each volume to obtain the correct final concentration.
9. Complete with sterile water to the final volume.
10. Inoculate the bacteria grown (Solution of step 5), previously washed and resuspended in 0.9% saline in the final volume of the formulation.

Final Formulation:

PEG (5, 10, 15, 20 and 25 weight / volume)
50mM Sodium Acetate
10mM Zinc chloride
Glycerol 0.2% vol / vol
Bacteria 0.1% (weight / volume)